ary changes after the divergence of the striped bass and zebrafish lineages may be responsible for the differential partitioning of activities among the *Hox* PG2 genes in those divergent lineages.

doi:10.1016/j.ydbio.2006.04.289

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Genetic analyses of adult pigment pattern development reveal homology and evolutionary novelty in *Danio* fishes

Margaret G. Mills, Richard J. Nuckels, David M. Parichy
Department of Biology, University of Washington, Seattle, WA, USA

Pigment patterns of *Danio* fishes are a convenient system for studying the evolution of development. In zebrafish, *D. rerio*, stripes form by migration and differentiation of distinct populations of melanophores: early metamorphic (EM) melanophores arise widely dispersed and then migrate into stripes, whereas late metamorphic (LM) melanophores arise already within stripes. EM melanophores require the kit receptor tyrosine kinase, as kit mutants lack these cells but retain LM melanophores that form a residual stripe pattern. To see if similar requirements are present in other species, we examined *D. albolineatus*, which has relatively few, uniformly dispersed melanophores. We isolated a null allele of *D. albolineatus* kit and asked whether residual, LM melanophores develop, as in *D. rerio*. We find that kit mutant *D. albolineatus* lack EM melanophores, yet retain LM melanophores. Interestingly, kit mutant *D. albolineatus* also develop a striped pattern similar to kit mutant *D. rerio*, indicating that (i) latent stripe-forming potential remains in this species, despite its uniform pattern; and (ii) evolutionary differences between *D. rerio* and *D. albolineatus* reflect changes in the behavior of kit-dependent melanophores, which migrate into stripes in *D. rerio* but fail to do so in *D. albolineatus*. Our results show how genetic analyses of closely related species can reveal both conservatism and innovation in developmental mechanisms, and the cellular processes underlying evolutionary changes in adult form.

doi:10.1016/j.ydbio.2006.04.290

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Characterization of zebrafish Deltex homologues

Stephanie, Chia-Chia Tan, Haoying Xu, Yun-Jin Jiang
Laboratory of Developmental Signalling and Patterning, Institute of Molecular and Cell Biology, 61 Biopolis Drive, Singapore

It is widely known that Deltex is a cytoplasmic protein that binds to Notch and may mediate CSL-independent Notch signaling. Deltex proteins share three functional domains: the WWE domain that binds to Notch ankyrin repeats, the Ring Finger (RF) domain that is often found in a subset of E3 ubiquitin ligases and a proline-rich region. We have cloned three full-length zebrafish *deltex* homologues, namely *deltex1*, *deltex2* and *deltex3*. In silico domain analysis revealed structural similarities as well as differences among the zebrafish *deltex* homologues. The most interesting difference is the lack of a WWE domain in Deltex3, which indicates that it may not interact with Notch receptors directly. Whole mount in situ hybridization assay demonstrated zebrafish *deltex1* expression in many tissues, including neural and sensory structures, raising the possibility that it may be involved in neurogenesis via the Notch signaling pathway. Increasing evidence suggests that Deltex possesses an E3 ligase activity and is responsible for endosomal trafficking of Notch through interaction with the Notch ankyrin repeats. The E3 ligase activity of zebrafish Deltex1 was carried out by in vitro ubiquitylation assay. Our result confirms that Deltex1 has an E3 autoligase activity in the presence of an E2, UbcH5a. Our characterization provides the first description of expression pattern of Deltex homologue and demonstration of its E3 ligase activity in zebrafish that would help in determining the molecular function of Deltex in the context of Notch signaling.

doi:10.1016/j.ydbio.2006.04.291

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A zebrafish Pax6a reporter BAC recapitulates Pax6 expression in the mouse

Jorn Lakowski, James D. Lauderdale
Univ. of Georgia, Athens, GA, USA

Pax6 is a highly conserved transcription factor which is crucial to the development of the central nervous system, eye and pancreas. Pax6 transcription is complex and subject to very strict regulatory mechanisms, which include a large number of tissue specific regulatory elements as well a differential promoter usage. Unlike most vertebrates, zebrafish have two Pax6 genes, designated Pax6a and Pax6b, which are located on different chromosomes, and likely arose from a genome duplication that occurred after the split between the tetrapod and teleost lineages. The Pax6 proteins encoded by Pax6a and Pax6b share 95% amino acid identity over their entire length and both generate ectopic eyes in *Drosophila* suggesting that the two proteins have retained similar biochemical functions. Furthermore, it has been postulated that both genes have been retained due to a partitioning of certain tissue specific, regulatory elements, crucial to proper Pax6 function. In order to test the degree of evolutionary conservation of the mechanisms governing Pax6 expression as well as to further investigate the basis for the retention of two Pax6 genes in fish, we took advantage of BAC modification technology. We have tested a dual reporter BAC containing the zebrafish Pax6a transcription unit (200 kb) in both mouse and zebrafish and demonstrate that the teleost regulatory elements can in fact direct reporter gene expression in the mouse. Our findings directly show that the factors occupying distinct regulatory elements can induce proper Pax6 transcription despite the vast

evolutionary distance between the two species and suggest that the mechanisms controlling Pax6 are conserved between mouse and fish.

doi:10.1016/j.ydbio.2006.04.292

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Cis-regulation of the Pax6 gene in the ascidian _Ciona intestinalis_

Vera C. Fonseca, Steven Q. Irvine
Univ. of Rhode Island, USA

Sequence analysis comparing the Pax6 region of *Ciona intestinalis* with that of *Ciona savigny* reveals conserved elements in two large introns in addition to conserved elements upstream of the first exon. This pattern is reminiscent of the locations of *cis*-regulatory elements in vertebrates. However, very little sequence conservation is found between ascidians and vertebrates. We have constructed reporter transgenes to explore the *cis*-regulatory organization of Pax6 in C. intestinalis. This work will be extended to other ascidians to track the functional evolution of regulatory DNA in this gene.

doi:10.1016/j.ydbio.2006.04.293

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Analysis of a Pax group IV gene in the leech

Ian K. Quigley, Xuanhua Xie, Marty Shankland
University of Texas, Austin, Texas, USA

Pax group IV genes encode transcription factors that play important roles in animal development. Although extensively characterized in certain model system species, the Pax group IV genes of Lophotrochozoa – one of the three great clades of bilaterian animals – have been minimally studied to date. Here, we report the cloning and expression analysis of a Pax group IV gene from a lophotrochozoan, the leech *Helobdella robusta*. Developmental RT-PCR suggests that *PaxIV-Hro* is not present as a maternal transcript but is expressed zygotically during segmentation. By combining lineage tracing with in situ hybridization, we demonstrate that early *PaxIV-Hro* expression in the leech is restricted to the N and O teloblast lineages, which contribute to the central nervous system. During organogenesis, *PaxIV-Hro* is primarily expressed in the supraesophageal ganglion and the segmental ganglia of the ventral nerve cord. *PaxIV-Hro* expression in the central nervous system is transient and decreases in a rostrocaudal direction as embryonic development proceeds. By further analyzing the expression, knockdown, and misexpression of *PaxIV-Hro* in the leech, we hope to elucidate both conserved and novel roles for this gene family in the Lophotrochozoa relative to the Deuterostomes and Ecdysozoans.

doi:10.1016/j.ydbio.2006.04.294

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Analysis of Pax genes during leech development

Matthew Schmerer, Marty Shankland
Univ. of Texas at Austin, Austin, TX, USA

Pax genes encode a family of transcription factors that play important roles in animal development. Although extensively characterized in certain model system species, the Pax genes of Lophotrochozoa – one of the three great clades of bilaterian animals – have been minimally studied to date. Here, we report the cloning and expression analysis of two Pax genes from a lophotrochozoan, the leech *Helobdella robusta*. Based on direct sequence comparisons and phylogenetic analysis, these two genes display an ambiguous affinity to Pax Groups I/II. One of these genes, tentatively named *Pax-beta*, is initially expressed as a maternal transcript that becomes localized during early segmentation, while the other, tentatively named *Pax-alpha*, initiates zygotic transcription during segmentation. In addition, both genes show broad patterns of zygotic expression during pattern formation and organogenesis. *Pax-beta* is primarily expressed in the nervous system, while *Pax-alpha* is expressed in foregut. By further analyzing the expression and function of these and other Pax genes in leech, our ultimate goal is to elucidate the ancestral function(s) and diversification of this gene family during the course of animal evolution.

doi:10.1016/j.ydbio.2006.04.295

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Evolutionary analysis of the _cis_-regulatory region of SM50, a gene that is essential for skeletogenesis in the sea urchin

Jenna L. Walters [1], Elaine M. Binkley [1], Ralph Haygood [2], Laura A. Romano [1]
[1] *Denison University, Granville, OH, USA*
[2] *Duke University, Durham, NC, USA*

An evolutionary analysis of transcriptional regulation will help us to understand the molecular basis of morphological diversity as well as provide critical insight into mutations associated with human disease. There is extensive morphological diversity in the larval skeleton of sea urchins. The SM50 gene encodes a secreted protein that is essential for skeletogenesis in the purple sea urchin, *Strongylocentrotus purpuratus*. We used PCR to amplify a portion of the SM50 gene (~1.5 kb) from genomic DNA obtained from 20 individuals of *S. purpuratus*. We then performed a variety of statistical tests to evaluate the mode and intensity of natural selection acting upon the *SM50* gene. Our results indicate that there has been directional selection on a region of DNA that is approximately 800 bp upstream of the transcriptional start site. Interestingly, this region of DNA does not include the non-coding sequence that is sufficient for *SM50* transcription during embryonic development in *S. purpuratus*. We also used PCR to amplify the *SM50* gene from genomic DNA obtained from the remaining nine species within the family Strongylocentrotidae.